### GW25-e1171

**Analysis of long non-coding RNA expression patterns in cardiac fat pads of canine with atrial fibrillation**

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**Objectives:** Long non-coding RNAs (lncRNAs) are indicated to be important orchestrators of gene regulatory networks. In this study, we aimed to characterize the lncRNA expression profiles in neural remodeling during atrial fibrillation (AF).
**Methods:** 6 adult beagle dogs of either sex were randomly divided into AF group (400 beats/min, right atrial pacing) and control group (without atrial pacing). After 4 weeks of tachypacing, the second-generation RNA sequencing was performed to examine the transcriptomes of lncRNAs in AF/ non-AF canine cardiac anterior right fat pads. The sequencing data were confirmed by quantitative real-time PCR (qRT-PCR). GO and KEGG pathway analyses were used to annotate the biological functions and pathways that the aberrantly expressed genes were involved in. Based on the sequence similarity, target genes of the lncRNA transcripts were predicted. Filtering pipelines were established to identify the candidate lncRNA transcripts.
**Results:** A sum of 61616 lncRNA transcripts was yielded by the high-throughout sequencing. Among them, 166 down-regulated and 410 up-regulated lncRNA transcripts with more than 2-fold change were identified, in which 45 transcripts were newly discovered in canine models of AF. 6 newly identified lncRNA transcripts were randomly selected and confirmed by qRT-PCR. Bioinformatic analysis showed that the aberrantly expressed genes were associated with neural growth, development, migration and neurodegenerative disorders. Additionally, based on differential expression levels, functions of the target genes, bioinformatic analysis and the tissue specific analysis, we selected two new lncRNA transcripts, TCONS_00032546 and TCONS_00026102, which might be involved in the process of neural remodeling by regulating their target genes at transcriptional level.
**Conclusions:** These results suggested that the dysregulated lncRNA transcripts might play a role in the initiation and development process of AF neural remodeling, which further provided potential therapeutic targets for prophylaxis and treatment of AF.

### GW25-e1408

**Hydrogen sulfide ameliorates High glucose-induced senescence by suppressing oxidative stress**

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**Objectives:** In patients with diabetes, the level of hydrogen sulphide ($H_2S$) is remarkably decreased. And it is demonstrated that endothelial senescence is accelerated under high glucose condition. The aim of this study is to investigate the effect of exogenous $H_2S$ on HUVEC senescence induced by high glucose.
**Methods:** Senescence model was established by treating HUVECs with 33 mmol/L glucose for 48 hours. Senescence was identified by β-galactosidase (SA-β gal) staining. MTT assay was used to assess cell proliferation. PAI-1, SOD1 and NF-κB p65 was analyzed by western blot. MDA level was measured using a commercial kit.
**Results:** High glucose induced a senescence-like phenotype in HUVECs as shown by slower proliferation, more SA-β gal positive cells and increased protein expression of PAI-1. In senescent cells, the SOD1 expression was reduced dramatically, but NF-κB p65 activity and MDA production was increased significantly. However, sodium hydrosulfide (NaHS, $H_2S$ donor, 100 μmol/L and 200 μmol/L) was able to promote cell proliferation, decrease the number of SA-β gal positive cells and reduce PAI-1 expression. In the meantime, NaHS increased SOD1 expression, inhibited the activity of NF-κB p65 and decreased MDA production.
**Conclusions:** Exogenous hydrogen sulphide prevents HUVECs against high glucose-induced senescence by modulating oxidative stress and NF-κB p65 activity. Our results may indicate that hydrogen sulphide treatment would be helpful to improve endothelial function in diabetic patients. Further studies are needed to explore the value of hydrogen sulphide in clinical practice.

### GW25-e2467

**Expression of Neutrophil Gelatinase-associated Lipocalin in Hypotonic Contrast-induced Rat Model of Renal Injury and The Effect of N-acetylcysteine on NGAL**

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**Objectives:** We built the rat model of contrast-induced nephropathy to observe kidney damage and the expression of neutrophil gelatinase-associated lipocalin (NGAL) in this situation. We also paid attention to the changes of NGAL level after the intervention of N-acetylcysteine (NAC), in order to know whether NGAL can be used as an index for early diagnosis of contrast-induced nephropathy (CIN), and whether NAC has renal protective effects.
**Methods:** Adult male SD rats of clean grade (total number 80) were randomly divided into four groups: control group (CON), contrast-induced nephropathy group (CIN), N-acetylcysteine group (NAC), and NAC plus CIN group (NAC+CIN). We collected blood samples and renal tissue of each group at the time point of 2h, 12h, 24h, 48h, and 72h after modeling (4 rats per time point of each group). Serum creatinine (Scr) values were measured by an automatic biochemical analyzer. Concentration of NGAL in serum was evaluated by Enzyme linked immunosorbent assay (ELISA) by using commercial kit. Immunohistochemistry and Western Blotting method is used to determine the expression of NGAL in renal tissue. HE-stained sections of rat kidneys were uses to assess the degree of kidney tubular injury. At the same time, renal oxidative stress was analyzed by MDA and T-SOD value.
**Results:** (1) Scr values: 2h, 12h and 24h after modeling, there showed no difference between the Scr values of CIN and CON group ($P>0.05$), 48h or 72h after modeling, Scr value was significantly increased in CIN group than in CON group or NAC+CIN group ($P<0.05$). There's no significantly difference between the Scr value of NAC+CIN group and CON group 48h after modeling ($P>0.05$), but difference appeared at the time point of 72h ($P<0.05$); (2) Kidney damage assessment of HE staining: 12h, 24h, 48h, 72h after modeling, different degrees of acute tubular injury occurred in CIN group, with or without epithelial cell brush border shedding, vacuolar degeneration, cell loss and regeneration, even part of tubular structural damage. At the time point of 12h, 24h, 48h or 72h, CIN group significantly showed more damage than CON group ($P<0.05$), and the damage scores of NAC+CIN group are higher than CON group too ($P<0.05$), but NAC+CIN group showed less damage than CIN group at the same time ($P<0.05$). Factorial analysis: The main effect of contrast agent was statistically significant ($F=64.128$, $P<0.01$). The interaction of contrast agents with NAC was statistically significant. (3) Serum NGAL lever: 2h, 12h, 24h, 48h or 72h after modeling, serum NGAL lever of CIN group is obviously higher than CON group ($P<0.05$), but that of NAC+CIN group is lower than CIN group ($P<0.05$). There was no difference between the serum NGAL levers of NAC+CIN group and CON group 2h after modeling ($P>0.05$). (4) Immunohistochemistry: 2h, 12h, 24h, 48h or 72h after modeling, IOD value of CIN group was significantly increased than CON group ($P<0.05$). (5) Western Blot: 2h, 12h, 24h, 48h or 72h after modeling, the NGAL level in kidney of CIN group was significantly increased than CON group ($P<0.05$). (6) Correlation analysis of tubular injury score and serum NGAL values shows that there's a positive correlation between them.
**Conclusions:** (1) The changes of NGAL level in both kidney and serum appears early in CIN rat model, and there's a positive correlation between tubular injury score and serum NGAL values. (2) NAC can reduce the renal tubular epithelial cell injury in CIN model, this effect may be produced through oxidative stress pathways.

### GW25-e3118

**Lin28a Protects Against Cardiac Ischemia/Reperfusion Injury in Diabetic Mice through the Insulin -PI3K-mTOR pathway**

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**Objectives:** The insulin-PI3K-mTOR pathway exhibits a variety of cardiovascular activities including protection against I/R injury. Lin28a enhanced glucose uptake and insulin-sensitivity via insulin-PI3K-mTOR signaling pathway. However, the role of lin28a on experimental cardiac I/R injury in diabetic mice are not well understood. The aims of the present study were to (1) determine whether lin28a protects diabetic mice from cardiac I/R injury and (2) identify whether the underlying mechanisms of lin28a is associated with the insulin-PI3K-mTOR dependent pathway.
**Methods:** Diabetic mice underwent 30 minutes of ischemia followed by 3h of reperfusion. Animals were randomized to be treated with lentivirus carrying lin28a siRNA (siLin28a) or control virus (siControl), lin28a cDNA (Lin28a) or control virus (Control vector) 72h before coronary artery ligation. Myocardial infarct size, cardiac function, cardiomyocyte apoptosis and mitochondria morphology in diabetic mice who underwent cardiac I/R injury were compared between groups. The target proteins of lin28a were examined by western blot analysis.
**Results:** Lin28a overexpression significantly reduced myocardial infarct size, improved left ventricular ejection fraction (LVEF), decreased myocardial apoptotic index and alleviated mitochondria cristae destruction in diabetic mice underwent cardiac I/R injury. Lin28a knockdown exacerbated cardiac I/R injury as evidenced by increased infarct size, decreased LVEF, increased apoptotic index and aggravated mitochondria cristae destruction. Interestingly, pretreatment with rapamycin abolished the beneficial effects of lin28a overexpression. Lin28a overexpression increased, while Lin28a knockdown decreased the expression of IGF1R, P-Akt, P-mTOR and P-p70s6k after cardiac I/R injury in diabetic mice. Rapamycin pretreatment abolished the effects of increased P-mTOR and P-p70s6k expression exerted by lin28a overexpression.
**Conclusions:** This study indicates that lin28a overexpression reduces infarct size, improves cardiac function, decreases cardiomyocyte apoptosis index and alleviates cardiomyocyte mitochondria impairment after cardiac I/R injury in diabetic mice. The mechanism responsible for the effects of lin28a is associated with the insulin -PI3K-mTOR dependent pathway.

### GW25-e3219

**Urotensin II induces endothelial-mesenchymal transition of cardiac microvascular endothelial cells via Smad2/3 activation**

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**Objectives:** Cardiac fibrosis is associated with the emergence of fibroblasts originating from endothelial cells through endothelial-mesenchymal transition (EndMT). The aim of the study was to explore the effect of UII on EndMT and its possible mechanisms.
**Methods:** Growth-arrested cardiac microvascular endothelial cells from neonatal rats were incubated in serum-free medium with UII ($10^{-8}$mol/l) and/or its receptor antagonist SB710411 ($10^{-6}$mol/l). To investigate the roles of Smad2, Smad3 in EndMT induced by UII, a small interfering RNA (smad2 siRNA or smad3 siRNA) were transfected into the cells. The phosphorylated Smad2/3 protein levels, α-smooth muscle-actin (α-SMA) and VE-cadherin induced by UII were evaluated by western blot. The CD31 were evaluated by flow cytometry.
**Results:** UII induced α- SMA expression in a dose-dependent manner, with maximal effect at a concentration of $10^{-8}$mol/l (23.4%). it decreased VE-cadherin expression in a dose-dependent manner, with maximal effect at a concentration of $10^{-7}$mol/l (91.3%). UII also significantly reduced expression of CD31. In addition, UII promoted Smad2/3 phosphorylation in a time-dependent manner, with maximal effect at 24h (811.6%). The effect was significantly inhibited by treatment with the UT inhibitor SB710411 ($10^{-6}$mol/l). Furthermore, Knockdown of Smad2 and Smad3 expression with siRNA significantly reversed the effect of UII.
**Conclusions:** Our data show for the first time that UII stimulates endothelial-mesenchymal transition,, which is mediated partly by the activation of the Smad2/3 signal pathways.

### GW25-e3239

### Geniposide Protects against Pressure Overload-Induced Cardiac Remodeling via 5'-Adenosine Monophosphate- Activated Protein Kinase-α

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**Objectives:** Cardiac remodeling featured as left ventricular dilatation, fibrosis, and impaired systolic function predisposes the affected individuals to heart failure. Geniposide (GE) is widely presented in traditional herbs possessed of anti-tumor effect. Whether GE protects pressure overload-induced remodeling has not been identified yet.
**Methods:** The mice were orally treated with GE (25-50mg/kg) for 7 weeks beginning at 1 week after thoracic aorta constriction (TAC). Mice only subjected to TAC or sham surgery as the control group received equal volume of vehicle. Echocardiography was performed to evaluate the chambers 8 weeks after TAC. The indices, namely body weight (BW), heart weight (HW), and tibia length (TL) were examined. HE and WGA staining were used to observe cross sectional area (CSA) of cardiomyocytes. Electron microscope was to scan myofilament and mitochondria. Hypertrophic markers were detected by RT-PCR. Western blot and immunofluorescence were applied to determine expressions of proteins. TUNEL was used to assay apoptosis.
**Results:** Decreased left ventricular diastolic diameter, restored ejection fraction and fractional shortening after GE administration were confirmed by echocardiography. Compared with sham, pressure overload resulted in increased HW/BW and HW/TL (4.28±0.25mg/g vs 8.06±0.55mg/g; 6.66±0.51mg/mm vs 12.09±1.21mg/mm) while GE restricted the elevated HW/BW and HW/TL in a dose-dependent manner (25mg/kg: 6.75±0.65mg/g for HW/BW, 10.57±1.09mg/g for HW/TL; 50mg/kg: 6.17±0.52mg/g, 9.31±0.45mg/g). After TAC, CSA were increased to 4.45 folds and GE treatment decreased CSA to 2.31 folds and 1.98 folds of sham, respectively. Electron micrograph showed loss of normal arrangement in the myofibrils and mitochondrial swelling. Moreover, 50mg/kg GE abolished increased atriopeptin, brain natriuretic peptide and β-myosin heavy chain to 9.3%, 43.7% and 18.9% of those in TAC group, respectively. Western blot showed that 50mg/kg GE inhibited phosphorylation of 5'-adenosine monophosphate-activated protein kinase-α (AMPKα) and suppressed activation of the downstream signal molecules including eukaryotic translation initiation factor 2-α kinase 3, glucose-regulated protein 78, glucose-regulated protein 94, and X-box binding protein 1. Translocation of nuclear factor-κB was detected by immunofluorescence. In addition, 50mg/kg GE improved imbalanced B-cell lymphoma 2/Bax and decreased cleaved caspase-3 induced by TAC. Apoptosis was also reduced by 50mg/kg GE confirmed by TUNEL.
**Conclusions:** GE protects pressure overload-induced cardiac remodeling via AMPKα.

### GW25-e3410

### Endothelial progenitor cells join in HHcy impaired angiogenesis

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**Objectives:** Abundant epidemiological and clinical studies have revealed the close relationship between hyperhomocysteinemia (HHcy) and CVD. During the last decade, we and others have demonstrated that HHcy can inhibit endothelial cell growth and postinjury reendothelialization, accelerate neointimal formation. However, the fundamental basis of endothelial progenitor cell in HHcy impaired angiogenesis remains unknown.
**Methods:** (1) Angiogenesis of HHcy mice under myocardial infarction. Cardiac function was measured with echocardiography (VisualSonics Vevo 770). Hearts were viewed in the short-axis and analyzed in M-mode. Changes in cardiac morphology and function (ejection fraction (EF) and fractional shortening (FS) ) were measured; Hearts were moved at 2 weeks/6 weeks after myocardial infarction and kept at -80°C. Frozen heart tissues were cut into 5μm thick slices. Adjacent sections were stained with rabbit polyclonal antibodies against CD31. Capillary density was expressed as $CD31^+$ endothelial cells per high-power field (200x). (2) Flow cytometry analysis. A volume of 200μl peripheral blood/bone marrow were incubated for 30 minutes in the dark with monoclonal antibodies against mouse vascular endothelial growth factor receptor 2 (VEGFR2) followed by PE-conjugated secondary antibody, with the APC-labeled monoclonal antibodies against mouse Stem cell antigen-1 (Sca-1). Each analysis included 100 000 events. The data were analyzed by LSR II flow cytometer (BD Biosciences, San Jose, CA). (3) MACS Separation-purify $Sca\text{-}1^+$ cells. Purity of $Sca\text{-}1^+$ cell is based on the use of MACS MicroBeads, MACS Columns and MACS Separators. Cells are initially immunolabeled with Anti-Sca-1-APC, after which magnetic labeling of $Sca\text{-}1^+$ cells can be achieved using Anti-APC MicroBeads. The cell suspension is then applied to a MACSColumn placed in the magnetic field of a MACS Separator. (4) Intravenous transfusions of Sca-1+ cells in HHcy MI mice angiogenesis. To evaluate the homing to infarcted heart of injected cells, 200μl purified $Sca\text{-}1^+$ cells were labeled with CellVueR NIR (near-infrared) 780 and injected periorbitally into C57/B6 mice, 6 hours before MI procedure. Images for tracing Sca-1+ cells were performed using the Multispectral FX Pro (Fixed Lens) Image Station at 0, 21, 24 and 45 hours. Strong fluorescence signals were observed at the heart. Cardiac function and capillary density checked after intravenous transfusions of Sca-1+ cells treatment.
**Results:** (1) HHcy impairs mouse cardiac function. Ejection fraction (EF) and fractional shortening (FS) were lower in HHcy mice group than control group, as well as heart capillary density. HHcy mice hearts have depressed function and less capillary density after myocardial infarction stress. Survival rate is also lower in HHcy mice. (2) Peripheral blood derived EPC percentage decreased in HHcy mice group and bone marrow derived EPC percentage is higher in HHcy mice group, but cell death rate is also higher in HHcy mice. (3) Intravenous transfusion of $Sca\text{-}1^+$ cells treatment induce PB derived EPC percentage increase in both control mice group and HHcy mice group. 6 weeks survival rate increased from 12.5% to 27.3% in $hCBS^+Cbs^{-/-}$ cell treat group,and also 62.5% to 80% in $hCBS^+Cbs^{+/-}$ cell treat group; The LVEF increased from 19.3% to 38.5% in $hCBS^+Cbs^{-/-}$ cell treat group, and also 31.6% to50.9% in $hCBS^+Cbs^{+/-}$ cell treat group.
**Conclusions:** EPC joined angiogenesis after myocardial infarction which is so important to cardiac function. Cell treatment restores ischemia-induced angiogenesis in HHcy mice.

### GW25-e3438

### Atrial Fibrillation Electrical Remodelling via Ablation of the Epicardial Neural Networks and Suprathreshold Stimulation of Vagosympathetic Nerve

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**Objectives:** Vagosympathetic nerve stimulation and epicardial neural networks are important participants in atrial electrical remodelling (AER). Elucidation of the changes in the electrophysiological indicators of the atrial and pulmonary veins caused by epicardial neural network ablation and autonomic nerve stimulation may provide a theoretical basis for the clinical treatment of atrial fibrillation (AF).
**Methods:** A total of 13 beagle dogs were randomly divided into two groups: the control group ($n$=6), which was treated with a simple rapid atrial pacing (RAP) for 6 h, and the experimental group ($n$=7), which was treated with RAP+vagus nerve stimulation (VNS) for 6 h. Both groups were treated with epicardial ganglia plexus (GP) ablation after 6 h. The monophasic action potential (MAP), various parts of the effective refractory period (ERP) and AF induction rate were measured and recorded before and after pacing or ablation.
**Results:** With the extension of the pacing record time, the atrial MAP and ERP of the two groups shortened and AF induction rate increased in various sites ($P$ <0.05). Compared with control group, MAP and ERP shortened significantly, while atrial fibrillation inducing rate increased significantly at baseline and l h, 3 h, and 6 h after pacing in experimental group ($P$ <0.05). Following GP ablation, the atrial MAP, ERP and AF induction rate were not different from baseline levels ($P$ > 0.05).
**Conclusions:** Vagus nerve threshold stimulation exacerbated the deterioration of electrical remodelling, whereas the epicardial neural network ablation blocked or reversed the AER.

### GW25-e4195

### Influence of Allocryptopine on Reverse Cardiac Electrophysiological Abnormalities in Rabbits

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**Objectives:** Allocryptopine (ALL) is an alkaloid extracted from *Corydalis decumbens (*Thunb) Pers Papaveraceae. Several data point to the existence of its anti-arrhythmic effects, but the underlying mechanisms are unclear. The aim of this study was to investigate the influence of ALL on cardiac electrophysiology in rabbit heart.
**Methods:** Monophasic action potential (MAP) recording electrode and whole-cell patch clamp techniques was conducted to access the effects of ALL on epicardial